Degradation of atrazine and isoproturon in the unsaturated zone: a study from Southern England

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Abstract: The potential for degradation of atrazine or isoproturon in the unsaturated zone of two boreholes was studied under laboratory conditions. Intact and uncontaminated samples were obtained from regular depths of 0–16.45 m and 0–9 m using a percussion coring technique. The results showed that the deep unsaturated zone contained micro-organisms capable of degrading atrazine or isoproturon. The rate of degradation was much faster in surface soil than in most unsaturated materials of both boreholes. The amount of atrazine remaining six months after incubation also varied between the two boreholes. A relatively small amount of atrazine was lost from sterilised samples, suggesting a significant role for microbial degradation. The addition of nutrient and energy sources into materials of low degradation capacity did not enhance the degradation of atrazine. Degradation rate was more related to the presence of a competent microbial population rather than to the presence of indigenous organic matter. However, the competent micro-organisms are more likely to be present when the organic matter content is high. The type and activity of these micro-organisms and their physical environment may have considerable influence on atrazine degradation and are likely to be responsible for much of the variation in the rate of degradation observed at different depths.

Keywords: atrazine; isoproturon; unsaturated zone; subsurface; degradation

1 INTRODUCTION

Atrazine and isoproturon are used as selective herbicides for weed control in maize- and wheat-growing areas, respectively. Atrazine has also been used as a non-selective herbicide on non-cropped industrial land. Atrazine and isoproturon have been detected in drinking water sources in some regions of Britain.[1,2]

Recent studies have shown that atrazine and isoproturon can move rapidly through monolith lysimeters when water input exceeds a threshold of between 1 and 3 mm h^{-1}, which is expected on occasions in the UK.[3,4] However, these herbicides may not immediately reach the groundwater, and they may be susceptible to dissipation processes such as chemical and biological degradation during their movement through the sub-surface environment. Thus, the determination of the fate of these herbicides in the sub-surface environment is essential to estimate their potential to contaminate groundwater.

Herbicide degradation in surface soil has been studied by numerous workers, but very little is known of the degradation of herbicides such as atrazine or isoproturon in the unsaturated zone, especially under UK conditions.[5,6] The degradation of atrazine in subsurface and groundwater environments may proceed at rates that are considerably lower than those in surface soil due to lower microbial activity, nutrient availability, soil type and other environmental conditions such as temperature, aeration and moisture content.[7–10] Very little information is available on the degradation of isoproturon in sub-surface environments.[11–13] A comparative study in four European countries using percussion drilling apparatus succeeded in obtaining intact and uncontaminated samples from different depths in the unsaturated zone.[14,15]

Chemical degradation has been implicated in the dissipation of atrazine in soil and water.[16–20] However, most atrazine degradation studies report that no degradation occurs in the absence of micro-organisms.[21–23]

The present study investigated the potential for atrazine and isoproturon degradation in the unsaturated zone underlying agricultural land in the UK where these herbicides had been used and groundwater is an important resource for drinking water.

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2 MATERIALS AND METHODS

2.1 Borehole site

Two boreholes (SF1 and SF2) of 16.45 and 9 m depth, respectively, were installed in 1993 at Sonning Farm, Reading, UK. Samples of sub-surface core materials were taken from both boreholes at regular depths. The site where these boreholes were installed had been in continuous use for maize-growing for several years and had a history of atrazine use.

2.2 Obtaining samples

A 200-mm diameter core barrel fitted with a plastic liner was used to retrieve the sample.[8] The core barrel also comprised a core retainer, catcher and shoe. The core barrel was pushed into the ground using a percussion drilling apparatus to obtain undisturbed core materials from different depths. No water was injected into the borehole during the drilling to avoid any possibility of contamination. Once the core barrel was brought to the surface, the materials in the core retainer and shoe were discarded. The ends of the liner were sealed with caps and the cores transported to the laboratory for immediate processing. Sub-sampling of the materials for incubation studies was performed on the day of field sampling. The bottom part (5 cm) of the core was discarded. Sub-samples of the core materials were then aseptically removed from the centre of the liner using a sterilised spatula, well mixed and stored in sterilised containers at 3 °C for one week.

2.3 Physical and chemical analysis

Some of the physical and chemical characteristics of the materials taken from both boreholes were determined. The pH at each depth was measured using a pH meter and a mixture of 10 g materials and 25 ml 10 mM $CaCl_2$.[24] Organic matter was determined using wet oxidation by acid dichromate and then titration by ammonium ferrous sulphate.[25] The texture of materials was assessed using the finger texturing method.[24]

2.4 Total viable bacteria

Total viable bacteria for each depth were determined 24 h after the collection of core materials from the field by standard dilution plating and counting using 3 g litre^{-1} tryptic soy agar (TSA)[26] with three replicates. The number of colony forming units (cfu) was assessed seven days after incubation at 25 °C. No attempt was made to count the bacterial population after herbicide application. However, the possible presence of micro-organisms in the sterilised samples at the start of the study and at each extraction was checked using 3 g litre^{-1} TSA medium.

2.5 Treatments

Atrazine (99.9% purity, Greyhound Chromatography & Allied Chemicals, Merseyside, UK) or isoproturon (98.2% purity, Ciba Geigy Ltd, CGA 18731) stock solution of 500 mg litre^{-1} was prepared in methanol in a glass volumetric flask and stored at 2 °C. The required standards for HPLC (High Performance Liquid Chromatography) were prepared by serial dilution with acetonitrile in volumetric flasks. The herbicide solution used for the experiment was also prepared from the original stock with sterilised deionised water. An amount of the materials from different depths equivalent to 300 g (oven-dry basis) was placed in 1000-ml sterilised glass jars. Herbicide solution was added to the jars to obtain an initial concentration of 5 μg g^{-1} material. Triplicate samples were incubated in the dark at 25 °C. Samples of sterilised materials (autoclaved three times at 121 °C for 15 min) from each depth were also included. The samples were adjusted to the same water potential (90% of field capacity) which is adequate for microbial activity and does not promote anaerobic conditions. The jars were opened in a sterile workstation each week, sterile deionised water was added if necessary to maintain the moisture content of the samples, the materials were mixed thoroughly and returned for incubation. All of these operations were performed in a sterile workstation, using sterile solutions and sterile glassware.

2.6 Herbicide extraction

The degradation of atrazine in materials from borehole SF1 was determined by measuring the concentration of atrazine remaining in the samples 0, 1, 2, 3, 4, and 6 months after incubation. The amount of atrazine or isoproturon remaining in materials of SF2 was determined 0 and 6 months after incubation. Triplicate subsamples of 10 g were taken from each container for extraction using acetonitrile + water (90 + 10 by volume). A subsample of 10 g was shaken with 30 ml acetonitrile in centrifuge tubes overnight in the dark and then centrifuged for 10 min at 3000 *g*. The supernatant was filtered through a special hydrophilic filter nylon membrane (0.2 μm) which offers chemical resistance to acetonitrile. Preliminary tests showed that atrazine was not retained by these filters, and 100% recovery was obtained. A portion (1.5–2 ml) of the filtered extract was collected in glass vials for analysis. The remainder of the extracts and the standards used were stored at <4 °C in the dark after analysis for further analysis.

The efficiency of the extraction method and recovery of atrazine or isoproturon was evaluated by spiking these herbicides at three different concentrations into soil of different textures or different organic matter contents and into sterilised deionised water samples. The recovery of both herbicides was determined 3, 24, and 48 h after herbicide application into liquid and soil samples. These samples were incubated in triplicate at 2 or 25 °C.

2.7 Herbicide analysis

The concentration of atrazine or isoproturon was measured by HPLC using a UV detector at 220 nm after separation by reverse phase chromatography on an ODS(30) column, Ultracarb 5, 150 × 4.6 mm, and

a mobile phase of acetonitrile + water (70 + 30 by volume). The column was operated at constant room temperature (23 (±1) °C), and a mobile phase flow rate of 1 ml min^{-1}. The sample volume injected was 25 µl. The concentration of atrazine or isoprotruon in the extracts was determined twice in each vial under the same conditions.

2.8 Determination of atrazine residues in untreated materials

An immunoassay test (Millipore, 1991) was used for the detection of atrazine residues in the materials at levels lower than the detection limit by HPLC (10 µg litre^{-1}). Sub-samples were obtained from the centre of the core liner at each depth immediately after sampling in the field. These materials were extracted with acetonitrile as in the incubation experiments. The residue of atrazine was determined directly in the extracts using the immunoassay method with a Millipore Microwell Strip Reader (Millipore, 1991). Ten millilitres of the extracts were also taken in sterilised glass bottles and concentrated by evaporation under sterile conditions to 3 ml. The concentration of atrazine residue in the concentrated extracts was also redetermined using the immunoassay method.

2.9 Substrate addition

Sterilised bottles containing 50 g of fresh materials of low degradation capacity from 6.1 m depth were subjected to the following treatments: (a) control (unamended samples); (b) amended with 50 g of materials from 0.15 m, 9.15 m or 10.35 m depth, (c) amended with 50 g of materials from 0.15, 9.15, or 10.35 m depth which had been sterilised by autoclaving; or (d) amended with a sterilised solution which was extracted from materials from 0.15, 9.15, or 10.35 m depth. Surface (0.15 m) material and the sub-surface material from 9.15 m depth had high organic matter content and high degradation capacity, and the sub-surface material from 10.35 m depth had low organic matter content and low degradation capacity.

Soluble organic matter was extracted from 10 g surface or sub-surface materials with 20 ml pure water. The suspension was shaken at 25 °C for 24 h, and then centrifuged at 8000 *g* for 30 min. The supernatants were filtered through 0.2-µm filters into sterilised glass bottle. Fifteen millilitres of the extract solution, spiked with sterilised atrazine solution, was added to 100 g fresh soil materials. Fifteen millilitres sterilised atrazine solution containing only sterilised pure water was added to other control samples.

3 RESULTS AND DISCUSSION

3.1 Characteristics of core materials

The data in Tables 1 and 2 show that the chemical and physical characteristics of core materials from both boreholes varied with depth. For both boreholes, pH values were close to neutral throughout the profiles.

Table 1. Characteristics of core materials taken from borehole SF1

Depth (m)	Texture	pH	Organic matter (g kg^{-1})	Bacteria (log cfu g^{-1} soil)
0.15	Sandy loam	6.9	32.5	7.84
0.8	Sandy clay	7.8	3.7	6.91
1.6	Sandy gravels	7.7	1.8	6.08
2.2	Sandy gravels	7.5	0.2	4.83
2.6	Gravel	7.3	0	3.88
3.45	Clay	7.8	0.75	4.00
4.3	Clay	7.8	0.8	4.26
4.65	Silty clay	7.8	3.25	4.94
5.35	Clay	8.3	1.35	7.04
6.05	Silty clay	8.6	1.65	2.79
6.75	Silty clay	7.9	4.6	4.82
7.25	Clay	7.7	1.7	3.00
7.95	Clayey silt	7.9	4.1	3.60
8.55	Silty clay	8.1	6.2	2.20
9.15	Silty clay	7.7	15.1	2.51
9.75	Sandy clay	8	2.7	1.89
10.35	Sandy clay	7.8	1	2.58
10.85	Silty clay	7.8	6.4	3.66
11.65	Sandy silty clay	8	1.25	3.82
12.35	Chalk	8.7	1.55	7.11
12.9	Chalk	8.3	0.75	3.68
13.55	Chalk	8.2	0.1	3.82
14	Chalk	8.5	0.75	4.67
14.45	Chalk	8.7	0.7	2.94
15.45	Chalk	8.4	0.2	2.45
16.25	Chalk	8.1	0.35	4.69

Table 2. Characteristics of core materials taken from borehole SF2

Depth (m)	Texture	pH	Organic matter (g kg^{-1})	Bacteria (log cfu g^{-1} soil)
0.1	Sandy loam	7.1	33.47	7.99
0.3	Sandy clay	7.2	20.82	7.96
0.95	Sandy gravelly clay	7.7	1.39	5.83
1.68	Sandy clay	6.9	0.50	5.00
2.23	Sandy clay	7.8	1.65	5.26
2.88	Sandy silty clay	7.9	1.16	4.78
3.4	Chalk	8.6	0.99	3.08
4.2	Chalk	8.3	1.07	3.51
4.75	Chalk	8.2	1.20	2.78
5.3	Chalk	8.1	1.31	5.30
5.9	Chalk	8.7	1.54	5.08
6.7	Chalk	8.7	0.57	3.90
7.5	Chalk	8.6	1.48	3.68
8.25	Chalk	8.2	1.62	3.08

The organic matter content decreased with depth in both boreholes but high levels were detected in some sub-surface materials of SF1 from the layer (8–12 m) overlying the chalk. These high levels were not detected in the unsaturated zone of borehole SF2. The source of organic carbon in the deep sub-surface environments is probably the dissolution of organic matter of geological origin within the rock mass.[27] Bacteria in the unsaturated zone may grow most efficiently on carbon sources readily available from

indigenous sources.[28] All materials taken from regular depths from both boreholes contained viable bacteria, indicating that bacteria can survive in significant numbers in deep unsaturated environments. The number of these bacteria varied between depths and boreholes and was not related to the material type, pH values, or organic matter content. No microbial growth was observed in the plates inoculated with any of the sterilised samples at any sampling interval.

3.2 Methodology and quality control

The extraction method used in this study resulted in 95 (±2)% recovery of applied herbicides. No difference in recovery rates was found when the applied herbicide was extracted from materials with different textures or organic matter contents, 3, 24, or 48 h after application, nor were differences found between the recoveries of herbicides from soil samples at 2 or 25 °C incubation. The efficiency of the extraction method and the accuracy of the degradation protocol study have been confirmed in other laboratories.[14] Degradation rate was therefore based upon the recovery of atrazine or isoproturon at different times after herbicide application relative to the initial recovery.

3.3 Atrazine residues

The amount of atrazine residue detected in fresh materials from both boreholes is shown in Fig 1. These results show that atrazine was detected in the root zone and shallow sub-surface zone for both boreholes. No atrazine was detected in materials taken from zone deeper than 10.35 m depth. More atrazine was detected in surface materials of borehole SF2 than in those of SF1, but much more atrazine was detected in sub-surface materials of borehole SF1. The variation in atrazine residues with depth and between boreholes may indicate that dissipation with different rates might exist *in situ*. The amount of atrazine detected in the core materials was not related to the texture or organic matter content of the materials or bacterial population.

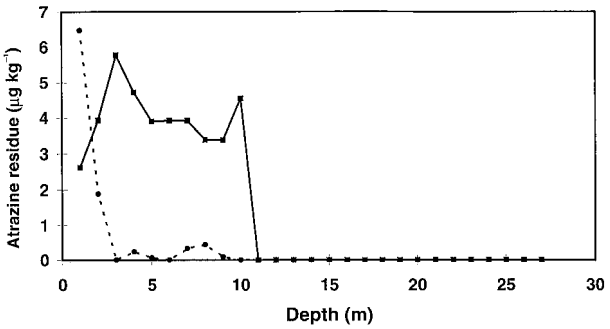

Figure 1. Atrazine residues in materials taken from boreholes (-■-) SF1 and (--●--) SF2.

3.4 Atrazine degradation in SF1

Degradation of atrazine was observed in all materials from borehole SF1 one month after adding atrazine (Fig 2). The amount of atrazine remaining in the materials after this time showed significant ($P = 0.005$) variation in the potential of these materials for atrazine degradation. The standard deviation among the replicates for all materials ranged from 7 to 16% at any sampling interval. Eighty percent of the applied atrazine was degraded in topsoil over a period of two months indicating a half-life ($t_{1/2}$) of less than two months. The half-life in sub-surface samples was significantly longer. However, in materials taken from 9 m and 10.8 m, 66 and 71%, respectively, of the applied atrazine disappeared during this period. In general, the materials overlying chalk (8 to 12 m depth) showed relatively high potential for atrazine degradation, with half-lives ranging from two to four months. However, in samples taken from within the chalk, more than 50% of the applied atrazine was recovered six months after addition.

The data in Table 1 and Fig 1 demonstrate that some sub-surface materials have a high bacterial population but show low potential for atrazine degradation. This suggests that the determination of total viable bacteria in sub-surface environments is not

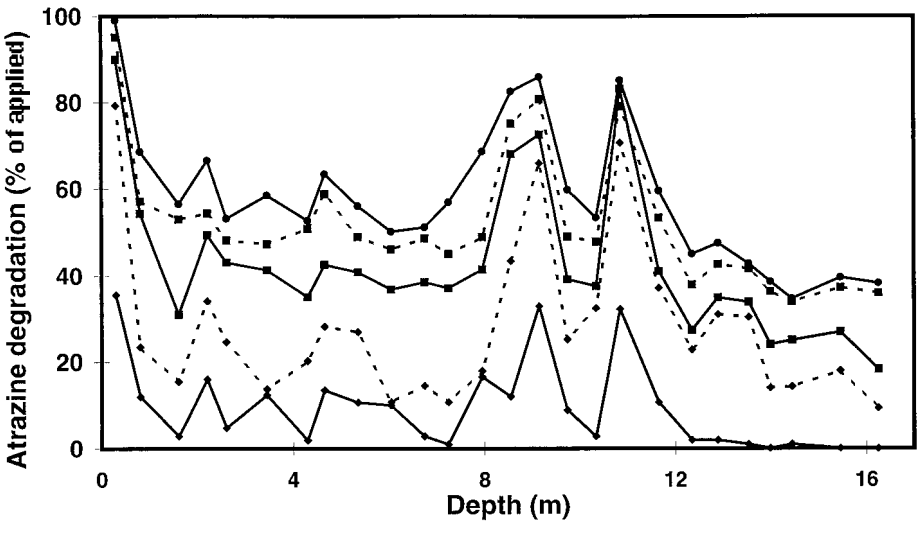

Figure 2. Degradation of atrazine (expressed as% of that applied) after (-◆-)1, (--◆--)2, (-■-)3, (--■--)4, and (-●-)6 months in materials from borehole SF1.

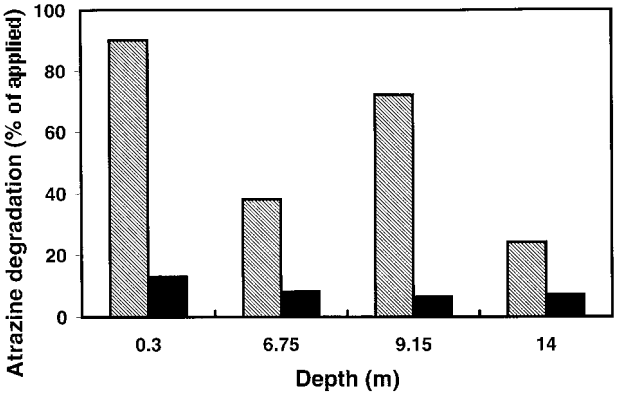

Figure 3. Degradation of atrazine (expressed as% of that applied) after three months in materials from borehole SF1 either (▨) untreated (fresh) or (■) sterilised.

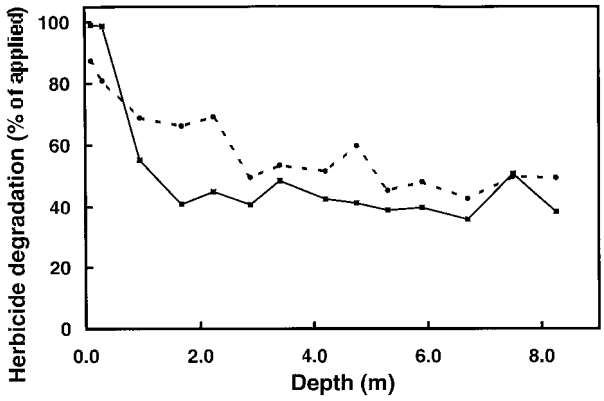

Figure 4. Degradation of (-■-) atrazine and (--●--) isoproturon (expressed as% of that applied) after six months in materials from borehole SF2.

sensitive enough to describe the influence of the microbial population on dissipation of atrazine. The degradation rate in some materials, such as those taken from a depth of 8.45 m, changed with time and increased more rapidly during the second month of incubation (30 to 60 days after incubation) than some materials which had the same or higher level of organic matter content. The data in Fig 2 also show that the potential for atrazine degradation two months after atrazine application was higher in chalk materials (12.25 m to 16.45 m depth) than in some clay materials. However, these clay materials showed higher potential for degradation during the last four months of incubation. These observations may indicate that some micro-organisms require more time than the others for the induction of specific enzymes or growth of an initially small degrading population, or a combination of both.[29] Neither material type (texture), total viable bacteria, nor the pH had a significant influence on the rate of degradation in this study.

3.5 Biological or chemical degradation?

Figure 3 shows the dissipation of atrazine in fresh and sterilised materials taken from different depths. Because the same pattern of degradation in fresh and sterilised materials was observed in all borehole materials, results of the degradation potential in four materials only are shown. Degradation of atrazine in fresh materials but not in sterile materials indicated that microbial involvement is responsible for the dissipation of atrazine in the materials used under our experimental conditions. The low rate of atrazine degradation in sterilised materials of different texture and levels of organic matter indicates a small effect of chemical degradation or long-term adsorption in the degradation of atrazine observed in this study.

3.6 Degradation of atrazine and isoproturon in SF2

Complete degradation of atrazine in the topsoil of borehole SF2 was observed six months after incubation (Fig 4). The rate of degradation decreased sharply with depth; 45 to 60% of the applied atrazine remained in the materials underlying the root zone (>1 m depth).

The rate of atrazine degradation in materials taken from deep locations in borehole SF2 contrasted with the degradation results determined in some sub-surface materials of borehole SF1. This demonstrates the variability in the potential for atrazine degradation between two soil profiles taken from the same site, and confirms previous work.[6] The organic matter content in the materials from borehole SF1, which showed high degradation capacity, was higher than in any sub-surface materials from borehole SF2.

The materials from borehole SF2 were also used to determine the potential of the microbial community to degrade isoproturon. The results (Fig 4) show that surface and sub-surface materials had the potential to degrade isoproturon. The rate of isoproturon degradation decreased with depth. Surface materials obtained from the top 0.6-m layer showed slightly higher atrazine degradation than isoproturon degradation. However, the degradation rate of isoproturon in sub-surface materials was higher than that of atrazine. There was no history of isoproturon use at the study sites.

3.7 Variability in sub-surface degradation

The high rate of atrazine degradation in materials taken from a depth of about 8 to 12 m from borehole SF1 was unexpected and contrasts with the findings of many workers.[12,30] However, it has been reported that some sub-surface environments may contain significant microbial numbers and activity.[31,32] Some of these micro-organisms may be capable of degrading contaminants and can thereby adapt to growth in the presence of these chemicals.[33] Previous work in our laboratory[5,6] also demonstrated atrazine degradation in samples taken from deep sub-surface environments.

It has been reported that the variation in the rate of atrazine degradation in two soil profiles was more related to the decrease in organic matter content and microbial population rather than to change of soil texture with depth.[30] Higher clay content does not necessarily protect atrazine against degradation, even

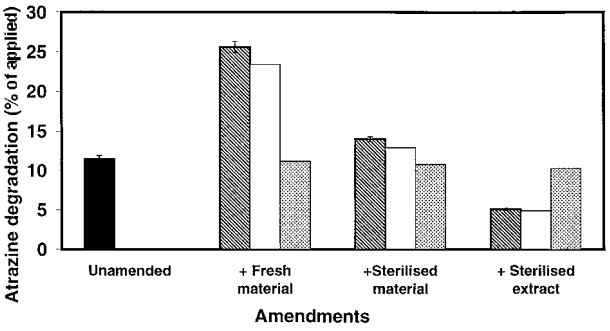

Figure 5. Degradation of atrazine (expressed as% of that applied) after four weeks in material from a depth of 6.1 m from borehole SF1 and amended with materials of either high degradation capacity (▨) 0.15 m and (□) 9.15 m depths) or (▒) low degradation capacity (10.35 m depth). Amendments were either (a) untreated (fresh) materials, (b) sterilised materials or (c) sterilised water extracts of materials.

though adsorption is usually higher in clay-texture materials than in those of lighter texture.[34] More atrazine was recovered in soils of low organic matter content than from soils with high organic matter content using 70+30 methanol+water.[35] This may suggest that the recovery of atrazine from materials with high organic matter is underestimated. However, it does not appear to be the case in our study. The difference is also not related to the fast hydrolysis of adsorbed atrazine by organic matter as suggested by Armstrong *et al.*[16] The degradation rate was not greatly related to the level of organic matter in the sub-surface zone of depth 8 to 12 m or to the clay distribution in the material.

It appears that the main factors controlling degradation rate in materials with similar organic matter contents are likely to be the population and activity of atrazine-degrading micro-organisms associated with the organic matter. The degradation rate may also be linked to the quality, rather than to the quantity, of organic matter present.[30] Although the presence of organic matter is essential for the survival and activity of micro-organisms in deep sub-surface environments, the influence of organic matter on atrazine degradation is not clearly understood. The considerable variation in the degradation potential of these sub-surface materials taken from different depths also indicates that substantial local variation in the size or activity of microbial population exists.[36]

In order to understand this, sub-surface material of low degradation potential was amended with different materials. The addition of fresh surface (0.15 m) or sub-surface (9.15 m) materials with high degradation capacity resulted in a significantly ($P<0.0001$) higher rate of atrazine degradation than that determined in unamended samples or in samples amended with sub-surface materials (10.35 m) with low organic matter content and low degradation capacity (Fig 5). The amendment with sterilised materials which contained high levels of indigenous organic matter and originally had high degradation capacity slightly increased the rate of degradation. However, the addition of a sterilised extracted solution containing a high content of soluble organic matter did not enhance, but reduced, atrazine degradation. In contrast, the addition of (a) a sterilised solution with low soluble organic matter or (b) sterile deionised water, did not result in any change in the potential for atrazine degradation. These data indicate that co-metabolizing micro-organisms are not active under these conditions. These observations suggest that atrazine was used in all core materials from both boreholes by micro-organisms as a source of nutrients (C and N) and energy.[37–40] The rate of atrazine degradation might therefore depend on the number of micro-organisms able to use atrazine as a sole source of C,N, or energy.

4 CONCLUSIONS

The absence of atrazine in SF1 at depths lower than 10.5 m could be as a result of high degradation capacity of the materials between 10.5 and 12 m, or the absence of atrazine movement into this zone. In SF2, run-off of atrazine because of the surface topography might have reduced the leaching into the lower zone. In neither case was the amount of residue detected related to the chemical or physical characteristics of the materials.

The results confirm that surface and sub-surface materials of both boreholes contained bacteria, and some populations of these micro-organisms were capable of degrading atrazine or isoproturon. The rate of degradation was in most cases much slower in sub-surface materials, due to the lack of degrading micro-organisms, but this varied from site to site. However, the localised high potential for herbicide degradation in the deep unsaturated zone was also found. The half-lives of herbicides under these conditions and therefore the potential for these compounds to contaminate groundwater are most likely governed by the type and activity of degrading micro-organisms rather than the amount of indigenous organic matter. However, as the high degradation capacity was associated with the presence of organic matter in sub-surface materials, it cannot be ruled out that the naturally occurring organic matter in the sub-surface environment might play an important role in the degradation of herbicides. The competent micro-organisms are also more likely to be present when the organic matter content is high.

The slow degradation rates in some sub-surface environments, even under optimum conditions for microbial growth and activity, suggest that the concentration of some herbicides in these deep environments is likely to take more than a decade to decrease significantly. These observations help to explain the detection of atrazine residues in some materials years after its last field application. The large amount of atrazine and possibly its breakdown products remaining in some sub-surface materials after six months' incubation indicates the potential for these chemicals to move to deeper saturated environments and act as a long-term source for contamination of groundwater.

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